Chemosystematics of *Juniperus*: Effects of leaf drying on the essential oil composition of *Juniperus pinchotii*

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ABSTRACT

A bulk collection of terminal branchlets was made from *J. pinchotii* and subjected to drying at 42ºC (24 hrs), then stored for up to 24 mos. at 22ºC (room temperature, RT). The oils were distilled and analyzed from fresh, 0.5, 1, 2, 4, 8, 16, and 24 mos. storage at RT. The oil yields showed a slight decline initially, but remained fairly constant. Camphor, camphene hydrate and citronellal declined (mg/g dry foliage) in fresh vs. 0.5 mo. samples. Borneol increased during storage (on a mg/g basis). This may be due to the loss of acetate by bornyl acetate and/ or oxidation of terpenes to produce borneol. Overall, most of the changes occurred between the fresh and 0.5 mo. samples. It appears one can use the oils from dried leaves of *Juniperus pinchotii* for geographical studies, but mixing fresh and dried leaf samples may present a problem for this taxon. Published on-line: **www.phytologia.org** *Phytologia 95(1): 10-17 (Feb. 1, 2013).*

KEY WORDS: *Juniperus pinchotii,* oils from dried leaves, chemosystematics, terpene decomposition.

With the importation of fresh plant materials into the USA (and other countries) becoming increasingly difficult due to plant quarantine laws, it is often necessary to utilize specimens that have frozen to kill insects and then air dried. However, the composition of the oils from air dried leaves may change during drying.

Achak et al. (2008, 2009) compared the leaf essential oils from fresh and air dried (22º C, 16 days) leaves for *J. thurifera* L., *J. phoenicea* L. and *J. oxycedrus* L. The first two species are in section *Sabina* and have scale-leaves, whereas *J. oxycedrus* is in section *Juniperus* with awl-like leaves (Adams, 2011). They reported small to moderate changes in several components, however, no statistical data were published.

Adams (2010) reported that the composition of *J. virginiana* leaf oils from specimens stored at room temperature (22º C) for up to 8 mos. were very stable. However, (Adams, 2011) later reported considerable differences in oil from *J. virginiana* leaves stored for 16 mos. Adams (2010) also examined the leaf oils of *J. pinchotii* from fresh and air dried for 2 weeks and found that oil yield declined from 1.45% to 1.10% (w/w, oven dry wt. basis). In addition, borneol increased and citronellal decreased (highly significantly) from fresh to 2 wk. at room temperature (22º C). Camphor significantly increased from fresh to dried leaves. The concentration of 4 other components changed from fresh to dried leaf oils (Adams, 2010).

The purpose of this study is to report on changes in composition of leaf oils from *J. pinchotii* leaves stored for long term (up to 24 mo.) at room temperature (22º C).

MATERIALS AND METHODS

Plant material - *J. pinchotii*, *Adams 12289*, 10 mi. s of Post on RR 669, Garza Co., TX. Voucher specimen is deposited in the Herbarium, Baylor University (BAYLU).

Isolation of oils - Fresh (200 g) and air dried (100 g) leaves were co-steam distilled with 20 mg of undecane (internal standard) for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (diethyl ether trap removed) with nitrogen and the samples stored at -20° C until analyzed. The extracted leaves were oven dried (48h, 100° C) for the determination of oil yields.

Analyses - The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software. For the comparison of oils obtained from leaves stored for various periods, associational measures were computed using absolute compound value differences (Manhattan metric), divided by the maximum observed value for that compound over all taxa (= Gower metric, Gower, 1971; Adams, 1975). Principal coordinate analysis was performed by factoring the associational matrix based on the formulation of Gower (1966) and Veldman (1967). Principal component analysis (PCA) follows Veldman (1967).

RESULTS AND DISCUSSION

Comparisons of the leaf components (on a mg/g foliage oven dry weight basis) from the leaves of *J. pinchotii* from fresh vs. air dried (42° C, 24 hr) then stored at room temperature (22°C) for 0.5 to 24 mo. are shown in Table 1. Of major interest are the changes in yield which varies little over the 24 mo. (Fig. 1). The yield appears to decrease from fresh to 0.5 mo., then shows an unusual decline at 2 mo. (Fig. 1). However, this may be due to sub-sampling. The leaf branchlets were pressed and stored in newspapers at RT. When the samples were distilled, entire branch--let, consisting of woody stems (up to ~ 3 mm cx.) with attached leaves were distilled. It may be that in the 2 mo. sample, a greater proportion of the woody stems were included. Because the oil is found chiefly in the leaves, not the wood, this could have led to a 'decline' in yield.

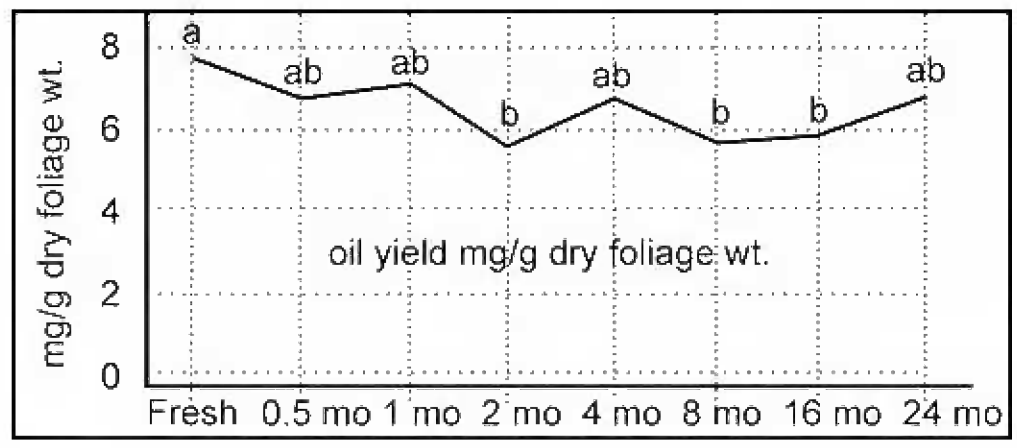

Figure 1. Variation in oil yield (mg/g) over a 24 mo. period. Any sample sharing a common letter is not statistically different.

The major volatile leaf oil components have highly significant differences (Table 1), except terpinolene (significant) and two components that were not significant (α- and γ-terpinene). Several compounds declined between fresh and 0.5 mo. drying (Fig. 2, camphor, camphene hydrate, citronellal). The decrease in camphor (Fig. 2) is significant, but subsequent changes are not significant. This pattern is also seen in camphene hydrate and citronellal (Fig. 2, Table 1). The spike in camphene hydrate at 4 mo. is unexplained.

Variation in monoterpene hydrocarbon components tended to have similar patterns (Fig.3). Limonene was significantly larger in samples from 1 mo. and 4 mo. and lower in 8 and 16 mos. (Fig. 3). γ-terpinene displayed no significant differences. Myrcene was stable with a decline after 4 mos. (Fig. 3). α-thujene increased from the fresh to 0.5 mo. samples and then displayed mostly steady concentrations (Fig. 3.).

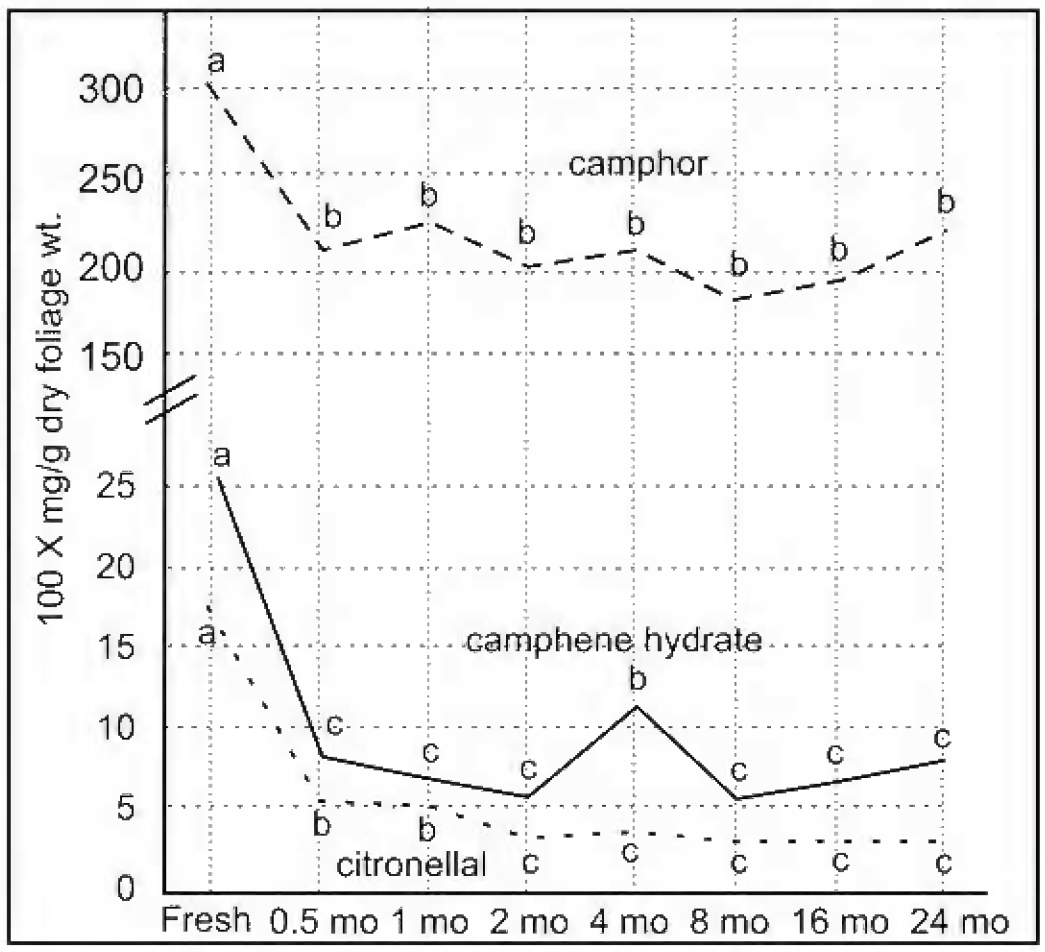

Figure 2. Variation in camphor, camphene hydrate and citronellal. Data points with different letters are significantly different. Data points with the same letter are not significantly different (P= 0.05).

One of the few compounds that increased was borneol (Fig. 4). This may, in part, be due to de-acetylation of bornyl acetate that declined (Fig. 4.). cis-sabinene hydrate declined initially, and then remained relatively stable (Fig. 4.).

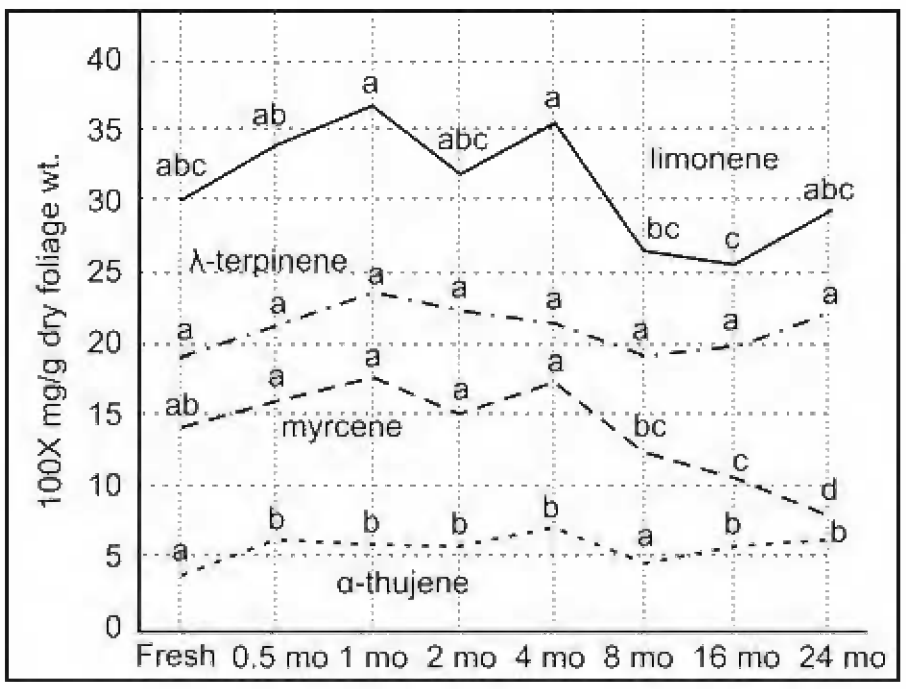

Figure 3. Variation among monoterpene hydro - carbons. Significance is as defined in Figure 2.

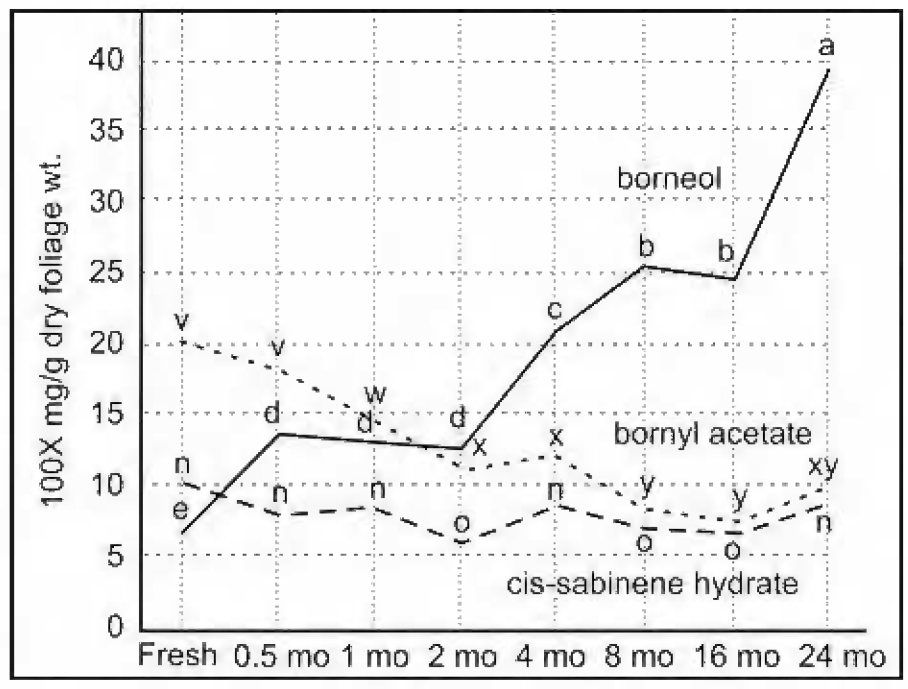

Figure 4. Variation in borneol, bornyl acetate and cis-sabinene hydrate. Note the inverse relationship between borneol and bornyl acetate.

Principal components analysis (PCA) of the 19 terpenoids and oil yields gave 3 eigenroots accounting for 31.1, 23.8 and 19.0% of the variance among these components. Plotting these three components reveals clustering by chemical classes except for oil yield, borneol, and bornyl acetate (Fig. 5). If borneol is increasing at the expense of bornyl acetate, that could explain their negative correlation (-0.69). Generally, the monoterpene hydrocarbons (C10-HC, Fig. 5) are in a group. Other groups are the oxygenated terpenes (C10-oxy, Fig. 5) and most of the sesquiterpene alcohols (C15-OH, Fig. 5).

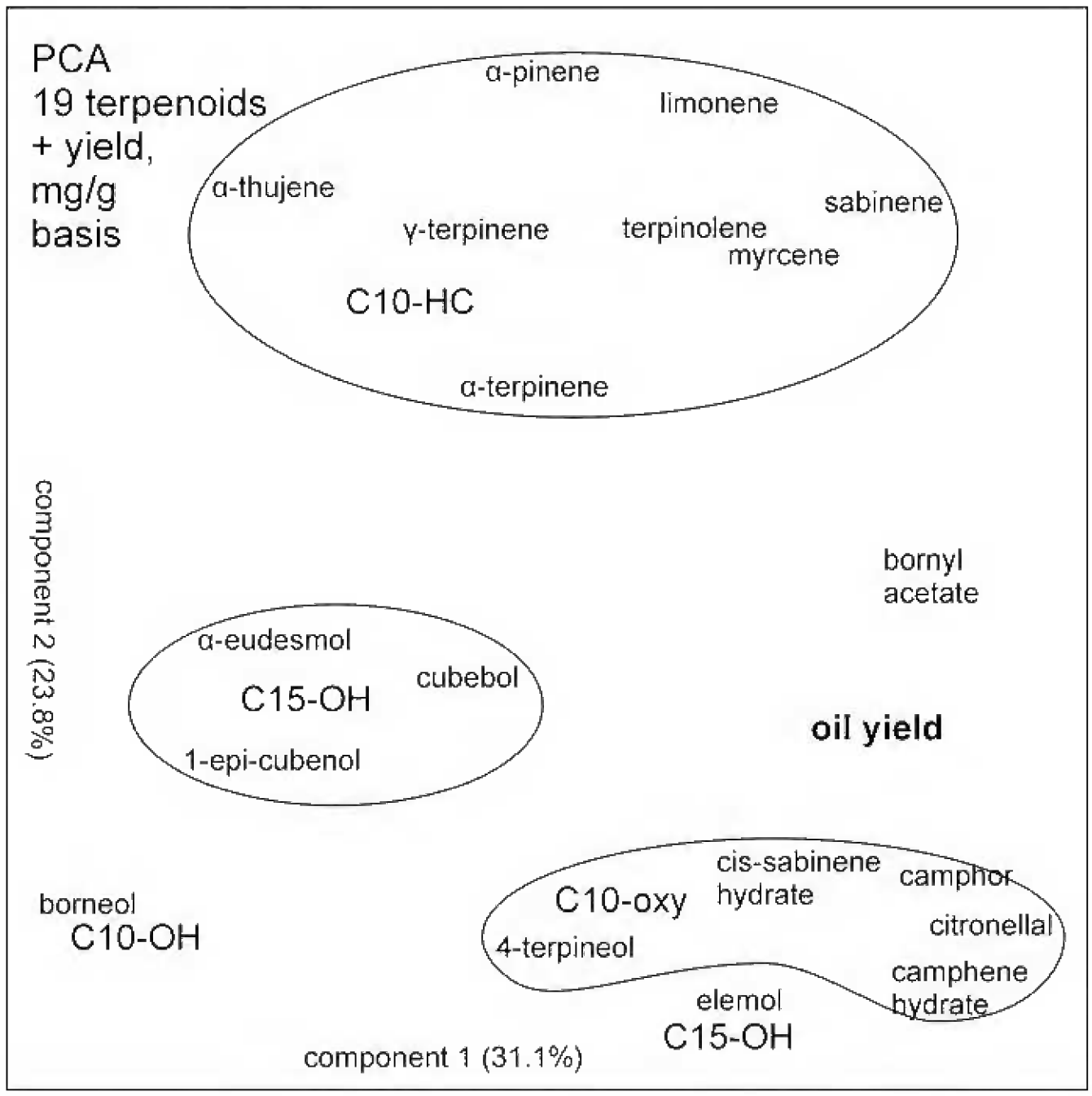

Figure 5. PCA of 19 terpenoids and oil yield (on a mg/g basis).

Juniperus pinchotii is in the serrate-leaf margined *Juniperus* group and has oil glands that rupture with white exudate on the leaves (Fig. 6). Fresh leaves were washed with diethyl ether and the wash compared with the oils from fresh leaves (Table 2). The major components of the ether wash were diterpenoids: sclareol, diterpene 2268, methyl abietate isomer, and an unknown diterpene acid (Table 2), none of these were found in the leaf oil. Camphor (40% in leaf oil) was 4.7% and bornyl acetate (2.7% in leaf oil) was 4.1%. The more volatile monoterpenes were absent or very small in the leaf wash, as one would expect from long-term exposure to ambient conditions. Several compounds absent in the leaf oil were found in the leaf wash: karahanaenone, p-cymen-8-ol, trans-sabinene hydrate acetate, trans-calamenene, sclareol, diterpene 2268, sempervirol diterpene acid 2408 and methyl abietate isomer (Table 2). It is not if the leaf glands ruptured, then sealed or continue to exude or 'bleed' components. It is interesting that not all of the leaves(Fig. 6) have ruptured glands. Gland rupturing may be a natural defense mechanism or a wound/ pathogen response.

Figure 6. Gland exudate in *J. pinchotii*.

The amount of changes between the oils from fresh and dried leaves of *J. pinchotii* (this study) is much greater than found in *J. virginiana* (Adams, 2010). To investigate the potential systematic use for the *J. pinchotii* oils, PCO was performed on the oils from the 8 storage tests and compared with oils from *J. ashei*. PCO ordination reveals that the *J. pinchotii* oils do cluster, but the fresh oil is somewhat different (Fig. 6). In addition, the 2 mo. oils show some differences (Fig. 6).

It appears that both the fresh and dry leaf oils of *J. pinchotii* could be used for chemosystematic studies involving *J. ashei* and likely other species. However, it also appears that for studies of geographic variation in *J. pinchotii*, either fresh or dried leaves could be used, but not both, as the differences may be large enough to mask geographical trends.

Clearly, additional studies would be useful to ascertain the changes found in the oils from fresh vs. air dried leaves stored for only 0.5 mo. These results are surprising, considering the mild drying and storage conditions used in this study.

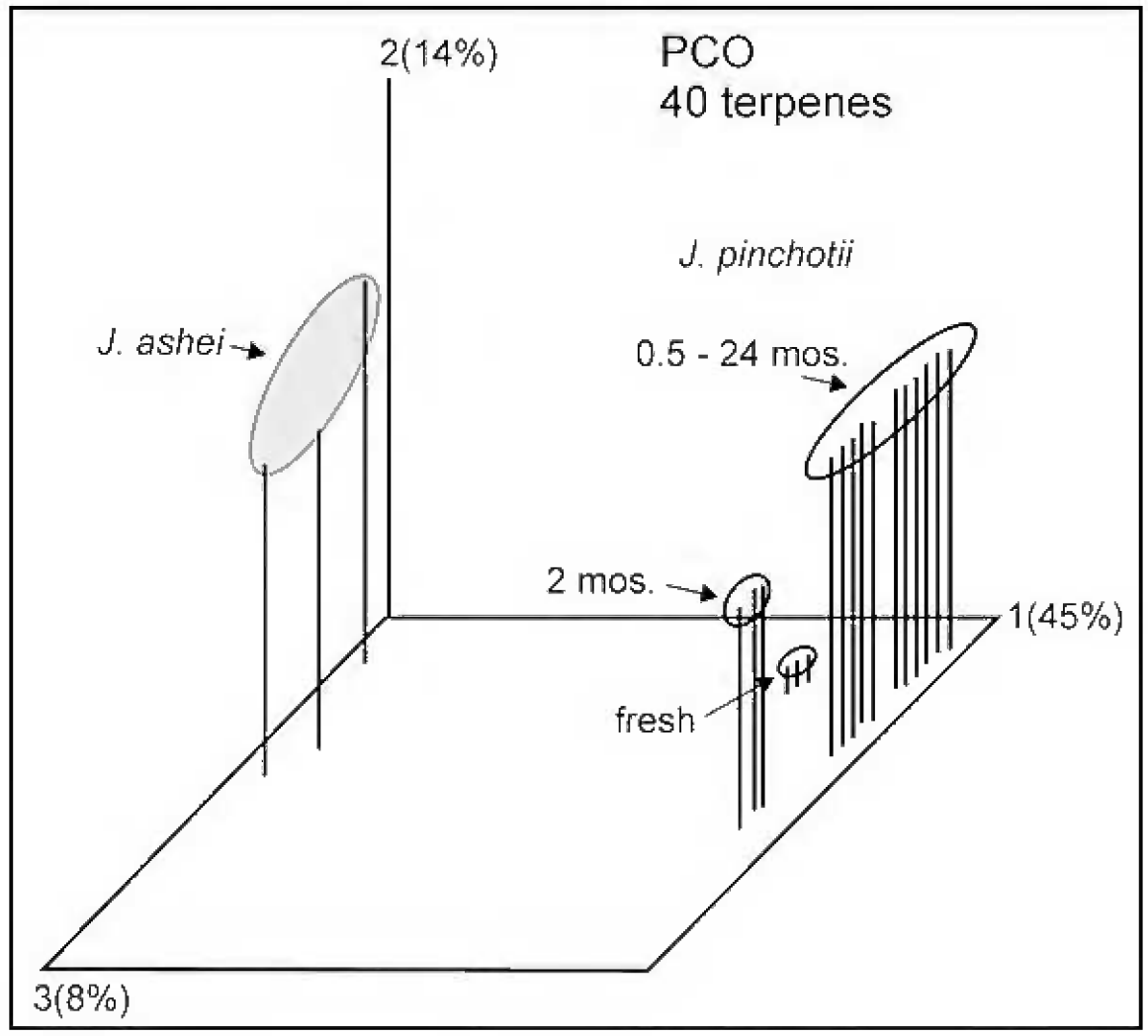

Figure 6. PCO based on 40 terpenoids for *J. ashei* and *J. pinchotii* stored for up to 24 mo.

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LITERATURE CITED

Achak, N., A. Romane, M. Alifriqui and R. P. Adams. 2008. Effect of the leaf drying and geographic sources on the essential oil composition of *Juniperus thurifera* L. var. *africana* Maire from the Tensift -Al Haouz, Marrakech region. J. Essential Oil Res. 20: 200-204.

Achak, N., A. Romane, M. Alifriqui and R. P. Adams. 2009. Chemical studies of the leaf essential oils of three species of *Juniperus* from Tensift Al Haouz-Marrakech Region (Morocco). J. Essential Oil Res. 21: 337-341.

Adams, R. P. 1975. Statistical character weighting and similarity stability. Brittonia 27: 305-316.
Adams, R. P. 1991. Cedar wood oil - analysis and properties. In Modern Methods of Plant Analysis: Oils and Waxes. Edits., H. F. Linskins and J. F. Jackson, pp. 159 - 173, Springler-Verlag, Berlin, Germany.
Adams, R. P. 2007. Identification of essential oils by gas chromatography/ mass spectrometry, 4th edition. Allured Publ., Carol Stream, IL, USA.
Adams, R. P. 2010. Chemosystematics of *Juniperus*: Effects of leaf drying on essential oil composition. Phytologia 92: 186-198.
Adams, R. P. 2011. The junipers of the world: The genus *Juniperus*. 3rd ed. Trafford Publ., Victoria, BC, Canada.
Gower, J. C. 1971. A general coefficient of similarity and some of its properties. Biometrics 27: 857-874.
Gower, J. C. 1966. Some distance properties of latent root and vector methods used in multivariate analysis. Biometrika 53: 326-338.
Veldman, D. J. 1967. Fortran programming for the behavioral sciences. Holt, Rinehart and Winston Publ., NY.

Table 1 Comparison of leaf oils (100 X mg/g basis) for major components obtained from fresh leaves of *J. pinchotii* vs. leaves dried and stored at 21° C for 0.5, 1, 2, 4, 8, 16 and 24 mos. ODW = oven dry wt. of extracted foliage. F sig = F ratio significance, P= 0.05 = *; P= 0.01 = **, ns = non significant, nt = not tested.

KI	Compound	Fresh	0.5 mo	1 mo	2 mo	4 mo	8 mo	16 mo	24 mo	F sig
	yield mg/g ODW	7.5	6.7	7.0	5.6	6.7	5.6	5.8	6.7	*
924	α-thujene 100X (mg/g)	3.6	6.0	5.8	5.7	6.7	4.5	5.5	5.9	**
932	α-pinene	5.6	8.1	8.1	7.0	8.5	6.4	6.0	6.2	**
969	sabinene	113	122	121	103	126	82.2	73.5	80.4	**
988	myrcene	14.2	16.6	17.6	15.0	16.8	11.9	10.8	7.6	**
1014	α-terpinene	11.5	12.7	14.2	12.9	13.1	11.4	11.6	12.8	ns
1024	limonene	30.0	33.6	36.7	31.9	35.2	26.5	25.2	29.5	**
1054	γ-terpinene	18.9	20.9	23.2	22.2	21.5	19.0	19.6	22.1	ns
1065	cis-sabinene hydrate	9.8	7.9	8.2	5.3	8.1	6.8	6.8	8.8	**
1086	terpinolene	7.5	8.2	9.2	8.1	8.3	6.8	6.9	7.9	*
1141	camphor	300	209	223	199	207	179	191	220	**
1145	camphene hydrate	24.5	8.0	6.7	5.8	10.9	5.4	6.3	7.6	**
1148	citronellal	17.0	5.4	5.2	3.2	3.4	3.0	2.8	2.8	**
1165	borneol	6.5	13.4	12.7	11.8	21.1	26.2	23.9	39.1	**
1174	terpinen-4-ol	54.0	44.2	51.9	42.0	53.3	45.5	52.9	61.6	**
1284	bornyl acetate	20.3	18.3	14.7	11.0	11.9	8.1	7.5	9.6	**
1514	cubebol	5.4	7.4	7.2	3.4	6.2	6.3	5.8	8.2	**
1548	elemol	10.6	8.0	9.3	4.8	7.2	7.1	8.3	9.5	**
1627	1-epi-cubenol	3.8	5.6	6.8	4.1	5.6	6.3	7.0	7.8	**
1652	α-eudesmol + α-cadinol	4.1	6.0	8.5	3.6	6.2	7.0	7.9	6.5	**

KI = Kovats Index (linear) on DB-5 column. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported.

Table 2 Comparison of components (percent total oil) from fresh leaves of *J. pinchotii* vs. ether wash of exudate.

KI	Compound	Fresh	ether wash
921	tricyclene	0.3	t
924	α-thujene	0.5	0.3
932	α-pinene	0.7	t
946	camphene	0.4	t
969	sabinene	15.1	0.9
974	β-pinene	0.1	-
988	myrcene	1.9	-
1002	α-phellandrene	t	-
1014	α-terpinene	1.5	t
1020	p-cymene	0.1	0.2
1024	limonene	4.0	0.2
1054	γ-terpinene	2.5	0.2
1065	cis-sabinene hydrate	1.3	0.4
1086	terpinolene	1.0	t
1098	trans-sabinene hydrate	0.2	0.5
1118	cis-p-menth-2-en-1-ol	0.4	-
1141	camphor	40.0	4.7
1145	camphene hydrate	3.3	0.2
1148	citronellal	2.3	-
1154	**karahanaenone**	**-**	**0.3**
1165	borneol	0.9	0.3
1174	terpinen-4-ol	7.2	0.2
1179	**p-cymen-8-ol**	**-**	**0.2**
1186	α-terpineol	0.4	0.3
1195	cis-piperitol	t	-
1207	trans-piperitol	0.1	-
1219	coahuilensol, me-ether	0.1	-
1223	citronellol	4.6	0.4
1253	**trans-sabinene hydrate acetate**	**-**	**0.2**
1274	pregeijerene B	t	-
1284	bornyl acetate	2.7	4.1
1451	trans-muurola-3,5-diene	0.2	0.3
1475	trans-cadina-1(6),4-diene	0.1	0.1
1493	trans-muurola-4,5-diene	0.4	0.4
1493	epi-cubebol	0.2	0.5
1514	cubebol	0.7	2.9
1521	**trans-calamenene**	**-**	**0.7**
1522	δ-cadinene	0.3	-
1548	elemol	1.4	0.8
1627	□-epi-cubenol	0.5	0.6
1630	γ-eudesmol	0.1	t
1649	β-eudesmol	0.2	0.3
1652	α-eudesmol + α-cadinol	0.6	0.3
1792	8-α-acetoxyelemol	0.2	0.6
1987	manoyl oxide	0.1	2.2
2055	abietatriene	t	1.2
2087	abietadiene	0.2	0.8
2222	**sclareol**	**-**	**13.0**
2268	**diterpene alcohol or aldehyde**	**-**	**2.1**
2282	**sempervirol**	**-**	**1.6**
2298	4-epi-abietal	0.2	3.1
2312	abieta-7,13-dien-3-one + abietal	0.3	7.2
2408	**diterpene acid**	**-**	**4.2**
2444	**methyl abietate isomer**	**-**	**5.4**

Table 3. Comparison of components (percent total oil) obtained from fresh leaves of *J. pinchotii* vs. leaves dried and stored at 21° C for 0.5, 1, 2, 4, 8, 16 and 24 mos. F sig = F ratio significance, P= 0.05 = *; P= 0.01 = **, ns = non significant, nt = not tested.

KI	Compound	Fresh	0.5 mo	1 mo	2 mo	4 mo	8 mo	16 mo	24 mo	F sig
	percent yield (% ODW)	0.75	0.67	0.70	0.56	0.67	0.56	0.58	0.67	*
921	tricyclene	0.25	0.48	0.35	0.48	0.50	0.64	0.35	0.36	nt
924	α-thujene	0.48	0.89	0.83	1.02	1.00	0.98	0.94	0.88	**
932	α-pinene	0.74	1.21	1.15	1.25	1.27	1.15	1.03	0.93	**
946	camphene	0.40	0.57	0.48	0.61	0.61	0.53	0.47	0.48	nt
969	sabinene	15.12	18.25	17.32	18.43	18.79	14.67	12.67	12.00	**
974	β-pinene	0.10	0.11	0.10	0.10	0.12	0.10	0.10	0.11	nt
988	myrcene	1.89	2.48	2.52	2.68	2.50	2.13	1.87	1.13	**
1002	α-phellandrene	t	t	t	0.20	t	0.10	t	t	nt
1014	α-terpinene	1.53	1.90	2.03	2.30	1.96	2.03	2.00	1.91	*
1020	p-cymene	0.10	0.36	0.34	0.47	0.47	0.66	0.64	0.62	**
1024	limonene	4.01	5.02	5.24	5.70	5.25	4.74	4.35	4.40	**
1054	γ-terpinene	2.52	3.12	3.32	3.79	3.21	3.39	3.38	3.30	*
1065	cis-sabinene hydrate	1.30	1.18	1.17	0.94	1.21	1.22	1.18	1.31	*
1086	terpinolene	1.00	1.23	1.31	1.44	1.24	1.22	1.19	1.18	*
1098	trans-sabinene hydrate	0.20	0.33	0.40	0.10	0.10	0.20	0.20	0.44	nt
1118	cis-p-menth-2-en-1-ol	0.38	0.42	0.54	0.47	0.41	0.46	0.57	0.62	nt
1141	camphor	40.01	31.15	31.80	32.51	30.96	32.02	32.85	32.91	*
1145	camphene hydrate	3.27	1.20	0.96	1.04	1.62	0.97	1.08	1.14	**
1148	citronellal	2.27	0.80	0.74	0.54	0.51	0.53	0.49	0.42	**
1165	borneol	0.86	2.00	1.82	2.11	3.15	3.60	4.12	5.83	**
1174	terpinen-4-ol	7.20	6.60	7.41	7.50	7.96	8.12	9.12	9.20	*
1186	α-terpineol	0.43	0.40	0.43	0.37	0.41	0.43	0.47	0.51	nt
1195	cis-piperitol	t	t	t	t	t	t	t	t	nt
1207	trans-piperitol	0.12	0.21	0.20	0.10	0.20	0.12	0.22	0.33	nt
1219	coahuilensol, me-ether	0.10	t	t	t	t	t	t	t	nt
1223	citronellol	4.62	3.30	3.67	3.14	3.17	3.40	3.59	3.23	nt
1274	pregeijerene B	t	t	t	t	t	t	t	t	nt
1284	bornyl acetate	2.71	2.73	2.10	1.96	1.78	1.44	1.29	1.43	**
1298	carvacrol	t	t	t	t	t	t	t	t	nt
1374	α-copaene	t	t	t	t	t	t	t	t	nt
1451	trans-muurola-3,5-diene	0.18	0.42	0.41	0.53	0.35	0.45	0.46	0.40	nt
1475	trans-cadina-1(6),4-diene	0.08	0.41	0.44	0.62	0.34	0.48	0.49	0.39	nt
1493	trans-muurola-4,5-diene	0.41	1.10	1.11	1.38	1.01	1.29	1.32	1.12	**
1493	epi-cubebol	0.19	0.29	t	t	0.33	0.33	0.34	0.33	nt
1500	α-muurolene	t	t	t	0.20	0.10	0.21	0.22	0.22	nt
1514	cubebol	0.72	1.10	1.03	0.61	0.93	1.12	1.00	1.22	**
1522	δ-cadinene	0.30	1.20	1.19	1.60	1.12	1.47	1.59	1.53	**
1528	zonarene	t	0.25	0.31	0.30	0.20	0.33	0.34	0.22	nt
1548	elemol	1.41	1.20	1.33	0.85	1.08	1.27	1.43	1.42	**
1627	1-epi-cubenol	0.50	0.84	0.97	0.74	0.84	1.12	1.20	1.17	**
1630	γ-eudesmol	0.08	0.33	0.41	0.44	0.60	0.42	0.45	0.46	**
1649	β-eudesmol	0.20	0.58	0.78	0.44	0.63	0.94	1.06	0.73	**
1652	α-eudesmol + α-cadinol	0.55	0.90	1.21	0.65	0.93	1.25	1.37	0.97	**
1670	bulnesol	t	t	t	t	t	t	t	t	nt
1792	8-α-acetoxyelemol	0.23	0.22	0.33	0.10	0.22	0.28	0.25	0.26	nt
1987	manoyl oxide	0.09	0.20	t	t	t	t	t	t	nt
2055	abietatriene	t	t	t	t	t	t	t	t	nt
2087	abietadiene	0.23	0.30	t	t	t	t	t	t	nt
2298	4-epi-abietal	0.21	0.40	0.49	0.11	0.37	0.48	0.49	0.59	**
2312	abieta-7,13-dien-3-one + abietal	0.33	0.58	0.88	0.27	0.59	0.77	0.78	0.92	**